

## Detection of Antioxidant and Antimicrobial Activities in Phenol Components and Essential oils of *Cistus ladaniferus* and *Mentha Suaveolens* Extracts

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The present study reports the phenol and flavonoid contents of *Cistus ladaniferus* and *Mentha suaveolens* from Northern Morocco and the evaluation of their in vitro antioxidant and antimicrobial activities against gram positive, gram negative bacteria, and yeast. The results showed that the highest phenol and flavonoid content found in the aqueous extract of *Cistus ladaniferus*. However, the methanol extract of *Mentha suaveolens* contains the high phenol and flavonoid contents. A significant anti-DPPH activity was obtained with aqueous extract of *Mentha suaveolens* and *Cistus ladaniferus* with IC<sub>50</sub> values of  $8.78 \pm 0.02 \mu\text{g/mL}$  and  $2.93 \pm 0.02 \mu\text{g/mL}$ , respectively. An important anticandidal effect of MSEO as observed against *Candida albicans*. The most sensitive bacteria to all extracts were *B. subtilis*, *P. mirabilis* and *S. aureus*. Our findings indicate that the natural compounds of *Cistus ladaniferus* and *Mentha suaveolens* with an antimicrobial activity could be a good target for the antioxidants and antimicrobial industry. However, further investigations regarding the isolation of phenolic bioactive compounds and the evaluation of their antioxidant and antimicrobial properties are required.

**Keywords:** Antioxidant Activity; Antimicrobial Activity; *Cistus Ladaniferus*; *Mentha Suaveolens*; Phenol Content.

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Since antiquity, plants form an important source of metabolite secondary to develop remedies to treat various diseases.<sup>1</sup> Recently, several scientific researchers have demonstrated that natural plant extracts possess biological properties such as antimicrobial, antioxidant, anti-diabetic, anticancer, and anti-inflammatory activities.<sup>2-7</sup>

*Cistus Ladaniferus* growing naturally in the Mediterranean region, namely in Morocco “Touzalt; touza'ch” frequently used in traditional medicines to treat many pathology such as stomachic problems and low libido case.<sup>8,9</sup> Several studies of the chemical composition of *Cistus Ladaniferus* essential oil (CLEO) indicate that

its main compounds were viridiflorol, p-cymene, camphene, bornyl acetate, pinocarveol,  $\alpha$ -pinene, and ledol.<sup>10</sup> Another work identified some flavonoids in the leaf of *C. Ladaniferus*.<sup>11</sup> In addition, a high content of viridiflorol, blumenol, limonene and spathulenol are detected in *C. Ladaniferus* methanol and ethanol extracts.<sup>12</sup> The biological effects of *Cistus Ladaniferus* as antibacterial, antioxidant, antifungal, and cytotoxic effects of *C. Ladaniferus* antifungal, are documented by several scientific works.<sup>13-17</sup>

*Mentha Suaveolens* (Lamiaceae family) locally known as "m'chichro" is traditionally used to treat cold, digestive system ailment, hemorrhoid, fever, intestinal swelling, flu against chill, and as carminative and conception and fertility booster.<sup>8,9,18,19</sup> The genus *Mentha* is largely distributed in temperate regions of the world.<sup>20</sup> *Mentha Suaveolens* was characterized by free flavonoid.<sup>21</sup> *Mentha Suaveolens* essential oils (MSEO) from different regions were characterized by high levels of menthone, piperitone oxide, pulegone, isomenthone, and piperitenone oxide.<sup>22</sup> The oils of many *Mentha* species exhibit an important biological proprieties.<sup>23-27</sup>

This study aims to determine the phenol and flavonoid contents of *C. Ladaniferus* and *M. Suaveolens* extracts and to evaluate the antioxidant and antimicrobial activity of these extracts, CLEO and MSEO.

## MATERIALS AND METHODS

### Plant Material

*Cistus Ladaniferus* and *M. Suaveolens* were collected from Taza region (006° 28, 382' E 004° 49,405' N and 34°10.510' N, 004°01.190' W, respectively) in 2016. The plants were identified in the laboratory of Natural Resources and Environment, Polidisciplinary Faculty of Taza, University Sidi Mohamed Ben Abdellah-Fez. The voucher specimens of plants were deposited FPT-LRNE-31 and FPT-LRNE-11, respectively. The collected material was dried in the laboratory at 25 °C under shade.

### Isolation of the Essential Oils

The extraction of CLEO and MSEO were carried out using hydro-distillation method. Indeed, the dried aerial parts (100g) of *C. Ladaniferus* were

and *Mentha Suaveolens* extracted in a Clevenger-type apparatus for 4 h. Then, CLEO and MSEO were stored at 4°C until use 3.

### Solvent Extracts

Dried powder (10g/100 mL) of aerial part of the plant was macerated with ethanol, methanol, and water at ambient temperatures. The extracts were filtered and concentrated using a rotary evaporator to obtain a dry extract; for aqueous extract the water was eliminated with the freeze-drying apparatus. All extracts were stored at 4-8°C until antimicrobial test was assessed.

### Estimation of Total Phenolic and Flavonoid Contents

In this work, TPC was estimated using the Folin-Ciocalteu method<sup>28</sup>. The Folin-Ciocalteu reagent 1/10 with methanol was prepared which 2.5 mL of this solution was mixed with 0.5 mL of a sample solution. Then 4 mL of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) (7.5 %) was added. After 30 min of incubation in water bath at 45°C the absorbance was measured at 765nm. TPC was expressed as mg of gallic acid equivalents per g of extract dry weight (mg GAE/g of extract).

The total flavonoid contents (TFC) in the extracts were revealed using the aluminum chloride method.<sup>29</sup> In brief, 0.3 mL of 5% sodium nitrite (NaNO<sub>2</sub>) was mixed with 5mL containing dissolved sample and distilled water. After 5 min, 0.3 mL of 10% aluminum chloride (AlCl<sub>3</sub>) was added and the solution was allowed to stand for 6 min. Then, 2 mL of 1 M sodium hydroxide (NaOH) was added and to reach 10 mL the volume was completed with distilled water. The solution was mixed vigorously and allowed to stand for 30 min. The absorbance was measured at 510 nm. The flavonoid content was determined as the rutin equivalent from the calibration curve of rutin standard solutions and expressed as rutin equivalent (mg RE/g of extract).

### Antioxidant Activity

#### Free Radical Scavenging Activity

The radical effect of EOs and extracts was evaluated using the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as reported by Huang *et al.*<sup>30</sup>, with some modifications. Briefly, the DPPH solution (0.2 mM in methanol) was prepared. Then 2.5 mL of test sample at different concentrations (2.5-100 µg/mL) were added to 0.5 mL of DPPH

solution and the absorbance of samples was measured at 517 nm after 30 min. Ascorbic acid and Trolox were used as positive controls.

Ascorbic acid and Trolox were used as positive controls. The calculation of the antioxidant activity was done according to the formula:  
DPPH scavenging activity (%) =  $[(A_0 - A_s) / A_0] * 100$

Where A<sub>0</sub> is the Absorbance of the negative control and A<sub>s</sub> is Absorbance of the test sample at 30 min. The test was carried out in triplicate and the IC<sub>50</sub> values were reported as means ± SD.

#### Reducing power of ferric ions

The reducing activity of EOs and extracts was determined according to the method of Oyaizu.<sup>31</sup> The mixture of the 1 ml the phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and the potassium ferricyanide (2.5 mL) was prepared. After incubation for 20 min at 50 °C (water bath), 2.5 mL of trichloroacetic acid (10%) was added to mixture. Then, the solution was centrifuged at 3000 Trs/min for 10 min. Finally, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm.

Ascorbic acid (50-450 µg/mL) is used as a standard. The reducing power is expressed in milligram equivalence of ascorbic acid per gram of Essential oil (mg AAE/g of EO).

#### Antimicrobial Activity

##### Microorganism Strains and Growth Conditions

To study the antibacterial activity of plant extracts, we have used the following bacteria *Listeria innocua* CECT 4030, *Staphylococcus aureus* CECT 976, *Bacillus subtilis* DSM 6633, *Proteus mirabilis*, *Escherichia coli* K12, *Pseudomonas aeruginosa* CECT 118, *Candida albicans* ATCC 10231 was the fungi used to evaluate the antifungal activity.

The pathogen bacterial strains were cultivated in Mueller-Hinton agar (MHA) or Mueller-Hinton Broth (MHB) at 37 °C for 24 h. The fungi were cultured in YPGA medium (5 g yeast extract, 5 g Peptone, 10 g Glucose, 15-18 g Agar, in 1 liter) or YPG, and incubated as follows: 48 h at 30 °C for fungi; The inoculum test concentrations are 106 CFU/mL for bacteria and 105 spores/mL for fungi.

#### Agar Disc Diffusion Method

The antimicrobial activity of *C. Ladaniferus* and *M. Suaveolens* extracts and EOs was performed using the agar disc diffusion method.<sup>32</sup> Briefly, sterile discs (6 mm diameter) containing 12.5 µL of pure essential oil or 20 µL of ethanolic, methanolic, and aqueous extracts at a concentration of 60 mg/mL dissolved by dimethylsulfoxide (DMSO) 10% were applied onto the surface of the agar-medium which was previously seeded by spreading by the inoculum concentrations test. After incubation, as described above, inhibitory zones were measured. Streptomycin 25 µg and DMSO 10% were used as positive and negative control, respectively.

#### Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration

The MIC values were determined in sterile 96-well microplate as described by Güllüce *et al.* 20, with some modifications. First, 100 µL of YPG was distributed in all test wells, except the first well in which a volume of 200 µL containing the essential oil or extracts with a concentration of 25 mg/mL in 10% DMSO. A series of concentrations ranging from 0.097 to 25 mg/mL were prepared by the transfer of 100 µL by scalar dilutions from the first to the ninth well. Then, except the 10th well used as sterility control, 10 µL of the suspension from each well was removed and replaced by the inoculum test concentration as described above. The eleventh well was considered as positive growth control containing only YPG broth. The last well containing 10% DMSO (v/v), without extracts, was used as negative control. Then, the plates were incubated at conditions of growth as described above. After an incubation, a volume of 25 µL of an indicator of microorganism's growth the was added in each well, the tetrazolium [MTT: 3-(4,5-dimethylthiazol)-2-yl-2, 5-diphenyltetrazolium bromide (Sigma)] prepared at a concentration of 0.5 mg/mL in sterile distilled water. The microplate was re-incubated for 30 min at temperature 25 °C. Where microbial growth was inhibited, the solution keeps the initial color of MTT. To determine the minimum bactericidal concentration (MBC) value, 10 µL of broth from the uncolored wells was inoculated in LPGA and incubated for 24 h or 72 h at 25 °C.

## RESULTS

### Estimation of Phenol and Flavonoid Contents

The estimation of phenol and flavonoid contents are expressed as mg of gallic acid or rutin equivalent per gram, respectively, was presented in Table 1. For *C. Ladaniferus*, The results showed that the aqueous extract is richer on TPC ( $76.98 \pm 4.6$  mg GAE / g of extract) compared to the methanol extract ( $59.8 \pm 4.99$  mg GAE/g of extract) and the ethanol extract ( $53.5 \pm 6.28$  mg GAE/g extract). However, TFC are only present the aqueous extract ( $35.51 \pm 2.04$  mg RE/g of extract). Concerning *M. Suaveolens*, the methanolic extract showed a high TPC with value of  $112.04 \pm 2.98$  mg GAE/g of extract), followed by the ethanolic extract whose content is in the range of  $101.07 \pm 4.34$  mg GAE/g extract, while  $92.70 \pm 5.82$  mg GAE/g extract are found in the aqueous extract. Furthermore, the content of the methanolic extract in flavonoids and of the order of  $99.77 \pm 5.4$  mg RE/g of extract, followed by a content of  $89.02 \pm 4.23$  mg RE/g of aqueous extract, on the other hand the content found in the ethanolic extract is  $68.66 \pm 2.94$  mg RE/g of extract.

### Antioxidant Activity

The results of antiradical activity of *C. Ladaniferus* and *M. Suaveolens* were summarized in Table 1. As we can see all extracts exhibit an

anti-DPPH in a manner of dose-dependent (Fig. 1 and 2). For *C. Ladaniferus*, the results showed that the aqueous extract exhibits a significant antiradical effect ( $IC_{50} = 2.93 \pm 0.02 \mu\text{g/mL}$ ), followed by methanolic extract ( $IC_{50} = 12.09 \pm 0.02 \mu\text{g/mL}$ ), and the ethanolic extract reduces the DPPH with IC 50 value of  $26.06 \pm 0.05 \mu\text{g / mL}$ . However, a low anti-DPPH effect was observed with MSEO with IC50 value superior to  $100 \mu\text{g/ mL}$ . For *M. Suaveolens* the results showed that the most important anti-free radical activity is caused by the aqueous extract  $IC_{50} = 8.78 \pm 0.02 \mu\text{g/ mL}$ , followed by ethanol extract  $IC_{50} = 12.57 \pm 0.03 \mu\text{g/mL}$ , and methanol extract  $IC_{50} = 15.83 \pm 0.05 \mu\text{g/mL}$ .

The results of FRAP assay, expressed as mg EAA/g, are summarized in Table 1. For *C. Ladaniferus* the aqueous extract possesses a significant reducing power of  $39.53 \pm 6.04$  mg EAA/g of extract, followed by the methanolic extract with a reducing power of  $23.69 \pm 0.48$  mg EAA/g of extract, and the ethanolic extract with  $21.08 \pm 0.06$  mg EAA / g of extract. One the other hand, among the extracts of *M. Suaveolens*, the methanolic and aqueous extract have significantly ( $p < 0.05$ ) a very significant reducing power  $54.44 \pm 5.52$  mg EAA / g of extract and  $53.03 \pm 1.65$  mg EAA/g of extract, respectively, compared to the ethanolic extract  $22.45$  mg EAA/g of extract.

**Table 1.** Extraction yield, phenolic content of *Mentha suaveolens* and *Cistus ladaniferus* and antioxidant activity of plant extracts

Extracts		Yield	TPC <sup>1</sup>	TFC <sup>2</sup>	DPPH IC <sub>50</sub> ( $\mu\text{g/mL}$ )	FRAP <sup>3</sup>
<i>Mentha suaveolens</i>	Ethanol	1.12 $\pm$ 0.02%	101.07 $\pm$ 4.34 <sup>a</sup>	68.66 $\pm$ 2.94 <sup>b</sup>	12.57 $\pm$ 0.03 <sup>ab</sup>	22.45 $\pm$ 1.12 <sup>a</sup>
	Methanol	2.63 $\pm$ 0.03%	112.04 $\pm$ 2.98 <sup>a</sup>	99.77 $\pm$ 5.04 <sup>a</sup>	15.83 $\pm$ 0.05 <sup>bc</sup>	54.44 $\pm$ 5.52 <sup>a</sup>
	Aqueous MSEO	3.37 $\pm$ 0.12%	92.70 $\pm$ 5.82 <sup>a</sup>	89.02 $\pm$ 4.23 <sup>a</sup>	8.78 $\pm$ 0.02 <sup>a</sup> >100	53.03 $\pm$ 1,65 <sup>b</sup> 5.48 $\pm$ 0.6 <sup>c</sup>
<i>Cistus ladaniferus</i>	Ethanol	5.67 $\pm$ 0.05%	53.5 $\pm$ 6.28 <sup>b</sup>	nd	26,06 $\pm$ 0.05 <sup>c</sup>	21.08 $\pm$ 0.06 <sup>b</sup>
	Methanol	6.24 $\pm$ 0.08 %	59.8 $\pm$ 4.99 <sup>b</sup>	nd	12.09 $\pm$ 0.02 <sup>b</sup>	23.69 $\pm$ 0.48 <sup>b</sup>
	Aqueous CLEO	6.64 $\pm$ 0.06%	76.98 $\pm$ 4.66 <sup>a</sup>	35.51 $\pm$ 2.04 <sup>a</sup>	2.93 $\pm$ 0.02 <sup>a</sup> >100	39.53 $\pm$ 6.04 <sup>a</sup> 0.1 $\pm$ 0.06

<sup>1</sup>TPC: Total Phenol Content expressed as mg GAE/g of extract.

<sup>2</sup>TFC: Total flavonoid Content expressed as mg RE/g of extract.

<sup>3</sup>Reducing power expressed as mg AAE/g of extract or EO.

MSEO: *Mentha suaveolens* essential oil

CLEO: *Cistus ladaniferus* essential oil

Data are reported to mean ( $n = 3$ )  $\pm$  SD.

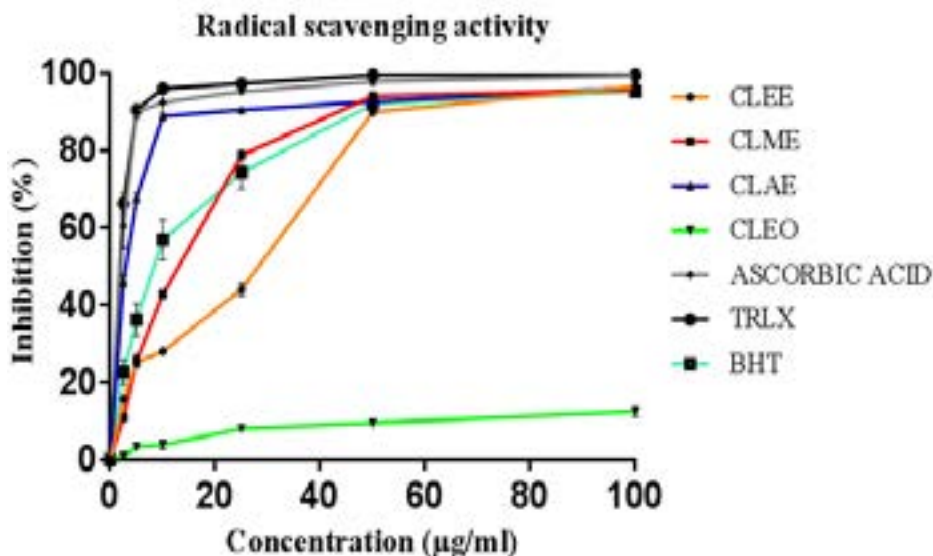
Values in the same row of plant column not sharing a common letter (a to c) differ significantly at  $p < 0.05$

nd : not detected.

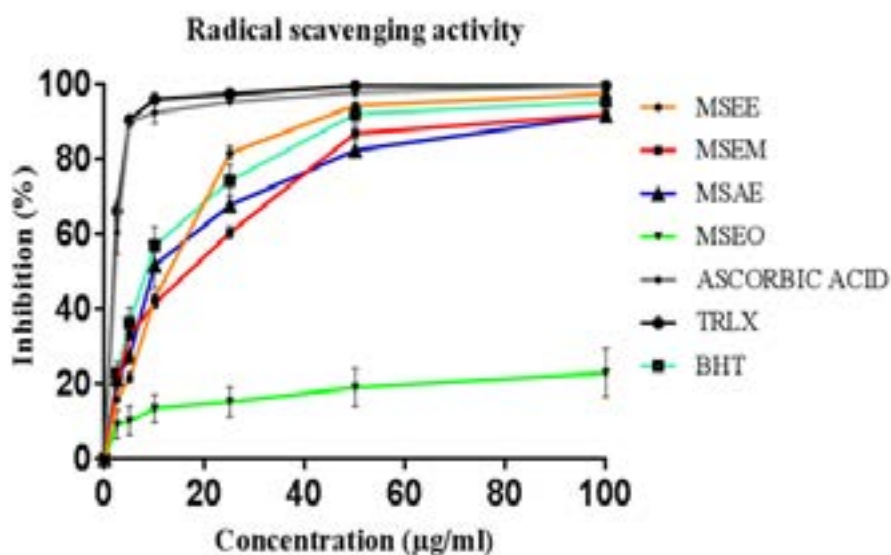
**Antimicrobial Activity**

The results of antimicrobial effects of extracts and EO of *C. Ladaniferus* and *M. Suaveolens* are expressed as diameters of inhibition and the minimal inhibitory concentration and listed in Table 2 and 3. On the hand, CLEO showed a remarkable antibacterial activity against

three bacteria tested with variable degree. Indeed, the most sensitive bacteria was *P. mirabilis* with diameter inhibitory zone value of  $25.66 \pm 3.05$  mm with MIC= 0.19 mg/mL, followed by *S. aureus* ( $22 \pm 2.64$  mm; MIC=6.25 mg/mL), and *B. subtilis* ( $15.33 \pm 1.52$  mm; MIC=0.78 mg/mL). However, a low antifungal was observed against



**Fig. 1.** Scavenging activity of *Cistus ladaniferus* extracts and essential oil (CLEE: *Cistus ladaniferus* ethanol extract; CLME: *Cistus ladaniferus* methanol extract; CLAE: *Cistus ladaniferus* aqueous extract; CLEO: *Cistus ladaniferus* essential oil ;TRLX: Trolox; BHT: Butylated hydroxytoluene)



**Fig. 2.** Scavenging activity of *Mentha suaveolens* extracts and essential oil (MSEE: *Mentha suaveolens* ethanol extract; MSEM: *Mentha suaveolens* methanol extract; MSAE: *Mentha suaveolens* aqueous extract; MSEO: *Mentha suaveolens* essential oil ;TRLX: Trolox; BHT: Butylated hydroxytoluene)

**Table 2.** Antimicrobial activity of *C.ladaniferus* and *M.suaevolens* essential oils of by disc diffusion method

	<i>C.ladaniferus</i>			<i>M.suaevolens</i>			Antimicrobial agents			
	EO	Methanol	Ethanol	Aqueous	EO	Methanol	Ethanol	Aqueous	Gentamicin	Ketoconazole
<i>S. aureus</i> CECT 976	22±2.64 <sup>a</sup>	19.66±0.57 <sup>b</sup>	20.33±0.57 <sup>b</sup>	-	19.33±1.52 <sup>a</sup>	-	-	-	34.33±0.57 <sup>c</sup>	nt
<i>B. subtilis</i> DSM 6633	15.33±1.52 <sup>a</sup>	10.66±0.57 <sup>b</sup>	15.33±0.57 <sup>a</sup>	-	28±2.64 <sup>a</sup>	10±1 <sup>c</sup>	10.33±0.57 <sup>c</sup>	-	25.66±0.57 <sup>c</sup>	nt
<i>L. innocua</i> CECT 4030	12±1 <sup>a</sup>	-	-	-	18.66±1.52 <sup>a</sup>	-	-	-	17.66±0.57 <sup>b</sup>	nt
<i>E. coli</i> K12	7.66±0.57 <sup>a</sup>	-	-	-	14.33±1.52 <sup>a</sup>	-	-	-	20.33±0.57 <sup>b</sup>	nt
<i>P. aeruginosa</i> CECT 118	-	-	-	-	11.33±1.52 <sup>a</sup>	-	-	-	19±1	nt
<i>P. mirabilis</i>	25.66±3.05 <sup>a</sup>	22.66±0.57 <sup>b</sup>	-	-	15.33±1.52 <sup>a</sup>	10.33±0.57 <sup>b</sup>	-	-	28.66±0.57 <sup>c</sup>	N3T
<i>C. albicans</i> ATCC 10231	10.66±0.57 <sup>a</sup>	18.33±0.57 <sup>b</sup>	16.66±0.57 <sup>c</sup>	-	49.66±0.57 <sup>a</sup>	-	-	-	20.66±0.57 <sup>b</sup>	

\*The diameter of the inhibition zones (mm), including diameter of disc (6 mm), are given as mean±SD of triplicate experiments; nt: not tested; -: no effect. Values of inhibition zone of same plant in line not sharing a common letter (a to c) differ significantly at p<0.05

**Table 3.** Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal (MBC) or fungicidal (MFC) Concentrations mg/ml of essential oils

Bacteria strains	<i>C.ladaniferus</i>			<i>M.suaevolens</i>			
	EO	Methanol	Ethanol	Aqueous	EO	Methanol	Aqueous
<i>S.aureus</i> CECT 976	6.25	0.78	>25	1.56	25	6.25	6.25
<i>B.subtilis</i> DSM 6633	0.78	12.5	-	3.12	25	0.78	12.5
<i>L.innocua</i> CECT 4030	-	-	-	-	-	6.25	6.25
<i>E.coli</i> K12	-	-	-	-	-	-	-
<i>P.aeruginosa</i> CECT 118	-	-	-	-	-	-	-
<i>P.mirabilis</i>	0.19	0.19	3.12	-	-	0.78	0.78
<i>C.albicans</i> ATCC 10231	-	12.5	>25	25	>25	1.56	12.5

-: no determined

*C. albicans* ( $10.66 \pm 0.57$  mm). For the crude extracts of *C. Ladaniferus*, the diameters of the zones of inhibition of the antimicrobial effect of the methanolic extract of *C. Ladaniferus* vary between  $10.66 \pm 0.57$  mm and  $27 \pm 00$  mm. Indeed, the methanol extract exhibits an important antibacterial effect against *Proteus mirabilis* ( $27 \pm 00$  mm; MIC=3.12 mg/mL), *Staphylococcus aureus* ( $20.33 \pm 0.57$  mm; MIC=0.78 mg/mL), with diameters of the zones of inhibition, of,  $19.66 \pm 0.57$  mm, respectively. *C. albicans* was inhibited with a diameter inhibitory zone of  $18.33 \pm 0.57$  mm with MIC value of 12.5 mg/mL. For the ethanol extract of *C. Ladaniferus*, the most sensitive bacteria was *S. aureus* ( $2.33 \pm 0.57$  mm; MIC=1.56 mg/mL), followed by *B. subtilis* ( $15.33 \pm 0.57$  mm; MIC=3.12 mg/mL). While, *C. albicans* was sensitive to ethanol extract with diameter inhibitory zone value of  $16.66 \pm 0.57$  mm and MIC value of 25 mg/mL. No activity was exhibited by aqueous extract of *C. Ladaniferus*.

On the other hand, the results indicate the interesting antibacterial activity of *M. Suaveolens* extracts and EO. In fact, *B. subtilis* was the most sensitive bacteria to the MSEO with diameter inhibitory zone of  $28 \pm 2$  mm and MIC value of 0.78 mg/mL, followed by *S. aureus* with  $19.33 \pm 1.52$  mm and MIC= 6.25 mg/mL, *L. innocua*  $18.66 \pm 1.52$  mm and MIC 6.25 mg/mL, and *P. mirabilis* with  $15.33 \pm 1.52$  mm and MIC=0.78 mg/mL. The MSEO exhibits a good antifungal activity against *C. albicans* with diameter inhibitory zone value of  $49.66 \pm 0.57$  mm and MIC value of 1.56 mg/mL. A low antibacterial activity was observed with ethanol and methanol extracts of *M. Suaveolens*. no effect of these extracts was observed against fungal tested.

## DISCUSSION

The results of our previous ethnopharmacological prospecting of medicinal plants conducted in the Province of Guercif (NE of Morocco) allowed us to appreciate and to know the practices of traditional herbal medicine transmitted by the population of the Province of Guercif, and have constituted a basis of selection of some plants to validate their uses as a remedy.<sup>8</sup> The *C. Ladaniferus* and *M. Suaveolens* were selected for the estimation of the phenol and flavonoid contents

and to evaluate the antioxidant and antimicrobial activities of their extracts and essential oils.

The present study indicates that the extracts of *C. Ladaniferus* are characterized by a high content of phenol content. These results are similar to previous studies that report a high phenolic content.<sup>33</sup> Contrary to our results, Amensour *et al.*<sup>12</sup> reported that the methanolic and ethanolic extracts of *C. Ladaniferus* have a lower phenol content of  $18.43 \pm 2.74$  mg GAE/g of extract and  $11.87 \pm 0.53$  mg GAE/g of extract. Concerning *M. Suaveolens*, the high phenol contents of this plant were previously reported.<sup>34</sup> Compared with plants of the genus *Mentha*, the results of other study indicates that the methanolic and aqueous extracts of the species *Mentha spicata*, *Mentha pulegium* and *Mentha piperita* are rich in phenol with contents which vary between  $167.2 \pm 0.41$  and  $305.4 \pm 1.32$   $\mu$ g TAE/mg including aqueous extract of *Mentha spicata* contains the highest phenol contents.<sup>35</sup>

The quantitative variation on the phenol contents may be attributed to some parameters such as the chemical structure, the method of extraction, the size of the particles forming the sample, and the solvent.<sup>36, 37</sup>

There is evidence in the literature that that the antioxidant activity correlates with the phenolic contents.<sup>38, 39</sup> Our results showed a dose-dependent percentage inhibition of DPPH reduction and a high reducing power of extracts of *Cistus Ladaniferus* in order aqueous > methanol > ethanol extracts; these results may be attributed to the presence of high phenol content in these extracts. Interesting antioxidant results are obtained for *M. Suaveolens*. Indeed, the aqueous extract showed the most significant anti-free radical activity, followed by the ethanol and methanol extract; knowing that the phenol-rich extracts are in the order methanol > ethanol > aqueous. The missing correlation between the phenol content and antioxidant activity could be explained by numerous factors; (i) the presence of non-phenolic products such as sugars, amino acids which reacts with Folin-Ciocalteu giving a high apparent phenolic level,<sup>40, 41</sup> (ii) the antagonistic interaction between phenolic and non-phenolic compounds,<sup>42</sup> (iii) the antioxidant activity depends on the numbers and position of functional groups.<sup>43</sup>

For the antimicrobial activity; on the hand, the *C. Suaveolens* extract and essential oil

were active against both gram- positive and gram-negative bacteria. The absence of antibacterial selectivity effect may be attributed to the presence of high content of products known for this propriety in the chemical composition of essential oil, and to the high phenol content in crude extracts. On the other hand, the gram positive strains were most sensitive to the essential oil of *M. Suaveolens* compared to the gram negative bacteria, these results may be attributed to the presence of phospholipids and lipopolysaccharides in Gram-negative membrane protected them against the external environment.<sup>44</sup>

### CONCLUSION

This study has displayed that extract of *C. Ladaniferus* and *M. suaveole* was possessed antioxidant and antimicrobial activities. Accordingly, we may need more achievement works in future to determine the specific active components which, are responsible for this antioxidant and microbial activity with suggestions for a mechanism of actions.

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